Appended General Laboratory methods

+

Extraction of DNA - Tissue is dissected from frozen or DMSO and sodium chloride preserved fish using a single edged sterile razor blade. DNA is extracted from the tissue by digestion in a mixture of CTAB and Proteinase K for approximately 4 hours at 55°C. Proteins are removed from the digest mixture by repeated extractions with phenol chloroform and the DNA is ethanol precipitated, dried and re-suspended in 10mM Tris (pH 8.5). This method has been used successfully to isolate DNA from a range of invertebrate and fish taxa (Dawson et al. 1998).

PCR amplification of D-loop DNA - For the tidewater goby, arrow goby and staghorn sculpin D-loop DNA is amplified from genomic DNAs using primers to the proline (CR-A) and phenolalanine (CR-M) tRNA's and standard PCR conditions (Lee et al., 1995). Attempts to use these primers on bay pipefish failed. However, D-loop DNA has been successfully amplified using primers to the tRNA threonine and conserved regions in the 12s gene.

Single Strand Conformation Polymorphism of D-loop DNA – Amplified D-loop DNAs are screened for unique genotype using a restriction digest and SSCP. Amplified D-loop DNAs are cut into small fragments (200-300 bp) using suitable restriction enzymes (Figure 2) and digesting at 37°C for 2 hours. To single strand the DNA, the digests are heated to 95°C for 3 minutes and immediately cooled on wet ice The samples are electrophoresed at 4°C in an 8% acrylamide/bis-acrylamide non denaturing gel. Migration patterns of the multiple digested strands are visualized using SYBR Green staining and the location of structural differences determined using a restriction map of the D-loop sequence.

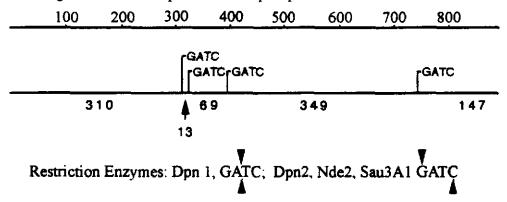


Figure 2. Restriction map of a tidewater goby D-loop indicating the restriction enzymes to be utilized for fragmenting D-loop sequences.

<u>Preparation of D-loop DNA for Sequencing</u> – Once the D-loop of an individual has been screened and shown to have a novel genotype, the PCR amplified DNA is isolated from the PCR mixture using QiaQuick PCR clean-up columns (Qiagen). The DNA is eluted into a small volume of 10mM Tris (pH 8.5) at high concentration and either manually sequenced or

sent to the DNA sequencing facility at California State University Northridge for automated sequencing.

Manual Sequencing of D-loop DNA - DNAs are manually sequenced using the Sequenase 2.0 DNA sequencing kit (Boehringer-Mannheim), and visualized with gel electrophoresis and autoradiography. The primers used to amplify the D-loop from fish DNA are used to directly sequence the PCR amplified D-loop DNA. The forward primer (CR-A) is used to sequence from the tRNA^{pro} end of the control region to the poly-T region and the reverse primer (CR-M) used to sequence from the tRNA^{phe} end of the control region. In cases where the amplified product is in low concentration, the DNA is cloned prior to sequencing using the TOPO-TA cloning kit (Invitrogen). These templates are sequenced using M13 Forward and M13 Reverse, universal primer sites located in cloning vector flanking the inserted DNA.

<u>PCR amplification of microsatellites</u> - The primers to amplify microsatellites from tidewater gobies have been provided to us by Holly Mendonca who identified them as a component of her research for a Master's thesis at San Jose State (see attached letter and curriculum vitae).

Locus	Repeating Unit	Product Size	No. of alleles
2	$(AC)_5AA(AC)_{10}$	152	2
8-1	(ATCTCT) ₅	146	3
12	(GT) ₁₂	133	3
14	$(GA)_7G(GA)_{11}$	233	3
22	Complex GT	281	Not analyzed

These primers are not highly variable, however to date they have been used to sample across only three closely spaced estuaries. We anticipate substantially more variation when they are applied to samples across the geographic range of tidewater gobies proposed in this study.

No specific primers are available to amplify microsatellites from arrow goby. However, our preliminary D-loop data support a close relationship between these putative sister taxa (C. Swift, pers. comm.). Arrow goby and tidewater goby sequences for the D-loop align smoothly with insertion of 2 single base gaps into the arrow goby sequence. The sequence exhibits 8% divergence from tidewater goby (compared to the 3% divergence within the tidewater goby), a reasonable divergence for closely related taxa. Thus, we will apply the primers developed for the tidewater goby to both the tidewater and arrow gobies. It should be noted that a number of studies of microsatellites in salmonids have successfully used microsatellite primers derived from other species and genera of salmonid fish (McConnell et al. 1995; Nielsen et al. 1997). In addition, a study of cross-species polymorphism reveals that microsatellite primer sites are maintained across widely divergent fish taxa and that locus heterozygosity is, in some cases, high across very divergent taxa (Rico et al., 1996). For example, the microsatellite *Gmo*02 derived from cod is highly polymorphic across 7 fish from 3 orders of teleosts as well as in sturgeon (Rico et al., 1996).

We have five microsatellite primer pairs for the bay pipefish, four of which have been provided by Adam Jones (Jones and Avise, 1997). These primers were previously used to assay parentage in the pipefish *S. scolvelli* where they were highly variable with 19 to 29

alleles per microsatellite. Although one of these primers sets is known to amplify in its congener, the bay pipefish (Jones and Avise, 1997a), the utility of the others has yet to be assessed. In addition, a microsatellite primer set from the stickleback *Gasterosteus aculeatus* has been shown to amplify in the bay pipefish (Taylor, 1998).

If the available primers prove inadequate for all or any of the three species in this study, we will have enriched microsatellite libraries constructed. An enriched library dramatically reduces the time required to screen for microsatellites (see attached letter from Ken Jones).

Analyzing PCR amplified microsatellites - Microsatellite samples will be run at the University of Texas Medical Center, Galveston in the laboratory of Dr. Lanzaro, who has extensive experience with microsatellite studies. His laboratory is equipped with an ABI 377 and robotics dedicated to microsatellite work. We are extremely pleased that he has agreed to collaborate with us on this work and feel his facility will allow the rapid recovery of high quality data. Our samples will be run against size standards and scored using protocols already in place in Dr. Lanzaro's lab (see attached letter and Lanzaro et al., 1998).

346 West Le Roy Avenue Arcadia, CA 91007-6909 28 February 1999

Dr. David Jacobs
Department of Biology
University of California
Los Angeles, California 90024

Dear Dave:

With this letter I would like to confirm my commitment to collaborate with you in working on the variation and distribution of the tidewater goby and related estuarine gobies along the California coast. As we have discussed, you, your students, and I have several mutual interests in the systematics of the species of bay gobies. My interest in the conservation, morphological variation, and systematics of these species should complement your laboratories interest in evolutionary dynamics at the molecular level. There is, of course, the natural extension of working on the related "Chasmichthys" species in Japan, particularly in unraveling the cross Pacific relationships.

As you know I already have current federal and state permits to collect the species for all of our purposes in California, and permits from the state parks systems where they are appropriate. My current work on several of the populations in southern California has resulted in some collections for biochemical purposes and I am sure the others can be obtained easily in the near future, most likely by the end of the summer of 1999.

The biochemical data to be obtained will undoubtedly contribute largely to the conservation strategy for tidewater gobies and for restoration of estuaries in California. This will be in addition to the interesting metapopulational questions obviously presenting themselves with the semi-isolated populations of this species.

Please let me know if you need any more information.

With best regards,

Camm C. Swift

Emeritus Associate Curator.

Natural History Museum of Los Angeles County

DEPARTMENT OF THE INTERIOR



1 PERMITTEE

FEDERAL FISH AND WILDLIFE PERMIT

2 AUTHORITY-STATUTES 16 USC 1539(A) 16 USC 1533(D)

50 CFR 17.32

REGULATIONS (Attached) 50 CFR 17.22

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11. CONDITIONS AND AUTHORIZATIONS:

- A GENERAL CONDITIONS SET CUT IN SUBPART D OF 50 CFR 13. AND SPECIFIC CONDITIONS CONTAINED IN FEDERAL REGULATIONS CITED IN BLOCK 42 ABOVE. ARE HEREBY MADE A PART OF THIS PERMIT. ALL ACTIVITIES AUTHORIZED HEREM MUST BE CARRIED OUT IN ACCORD WITH AND FOR THE PURPOSES DESCRIBED IN THE APPLICATION SUBMITTED. CONTINUED VALIDITY, OR RENEWAL OF THIS PERMIT IS SUBJECT TO COMPLETE AND TIMELY COMPLIANCE WITH ALL APPLICABLE CONDITIONS, INCLIDING THE FILING OF ALL REQUIRED INFORMATION AND REPORTS.
- 8. THE VALIDITY OF THIS PERMIT IS ALSO CONDITIONED UPON STRICT OBSERVANCE OF ALL APPLICABLE FORBIGN, STATE, LOCAL OR OTHER FEDERAL LAW.
- C. VALID FOR USE BY PERMITTEE NAMED ABOVE.
- D. Further conditions of authorization are contained in the attached Special Terms and Conditions.

ADDITIONAL CONDITIONS AND AUTHORIZATIONS ALSO APPLY

2 REPORTING REGULEMENTS

FIRST ANNUAL REPORT DUE: 01/31

See permit conditions for reporting requirements

SSUED BY

Lavoy Dolt

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CHEF - ENDANGERED SPECIES

DATE

09/29/1996

SPECIAL TERMS AND CONDITIONS FOR Dr. Camm C. Swift

- 1. You were previously issued this permit on November 15, 1996. The terms and conditions set forth in that permit are hereby superseded by this amendment.
- 2. Acceptance of this permit serves as evidence that the permittee understands and agrees to abide by the "General Conditions for Native Endangered and Threatened Wildlife Species Permits," 50 CFR Part 13, 50 CFR 17.22 (endangered species) and/or 50 CFR 17.32 (threatened species), as applicable (copies attached). In addition, the permittee must have any other applicable State and Federal permits prior to the commencement of activities authorized by this permit.
- Authorized to: take (capture and release, retain for lab studies, and sacrifice) the tidewater goby (Eucyclogobius newberryi); take (capture and release) the unarmored threespine stickleback (Gasterosteus aculeatus williamsoni); and take (harass by survey, capture and release) the California red-legged frog (Rana aurora draytonii) in conjunction with presence/absence surveys and scientific studies to enhance their survival as specified in the permittee's August 4, 1998, amendment request in accordance with the conditions stated below.
- Permitted activities are restricted to lagoons, estuaries, and other areas of suitable habitat in coastal counties of California between and including Del Norte County and San Diego County, except where otherwise specified below.

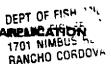
Proposals to conduct activities pursuant to this permit at specific locations within the above referenced areas must be submitted in writing to the appropriate Fish and Wildlife Office (FWO) of the Fish and Wildlife Service at least 10 days prior to conducting such activities. The appropriate FWO is determined as follows:

For Del Norte, Humboldt, and Mendocino Counties, contact the Coastal California Fish and Wildlife Office (CCFWO), 1125 16th Street, Suite 1209, Arcata, California 92008 (telephone: 707-822-7201; fax: 707-822-8411). For the Central Valley hydrographic basin and the coast ranges north of the Santa Cruz County line, contact the Sacramento Fish and Wildlife Office (SFWO), 3310 El Camino, Suite 130, Sacramento, California 95821 (telephone: 916-979-2725; fax: 916-979-2723). For areas from Santa Cruz County south to Los Angeles County north of the Angeles National Forest, contact the Ventura Fish and Wildlife Office (VFWO), 2493 Portola Road, Suite B, Ventura, California 93003 (telephone: 805-644-1766; fax: 805-644-3958). For areas from Los Angeles County including and south of the Angeles National Forest to San Diego County, contact the Carlsbad Fish and Wildlife Office (CFWO), 2730 Loker Avenue West, Carlsbad, California 92008 (telephone: 760-431-9440; fax: 760-431-9618).



DEPARTMENT OF FISH AND GAME -

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346 West Leroy Avenue Arcadia, CA 91007-6909 626 447-5846 Degrees: A. B. Zoology, U. C. Berkeley, M. A., Zoology, U. Mich, Ann Arbor, PhD, Fla. St. Univ, Tallahassee.

Academic Positions: (most recent first) Emeritus Assoc. Curator of Fishes, Nat. Hist. Mus. Los Angeles County, Visiting Asst. Prof., Biology, Loyola Marymount University, Los Angeles; Assoc. Curator Fishes, Natural History Museum of Los Angles County; simultaneously, Adj. Asst. Prof. Biology, Univ. S. California., and intermittently, Consultant in conservation biology and fisheries of southern California freshwater and estuarine fishes.

Research Interests: The biology, systematics, and paleontology of freshwater and estuarine fishes. Most recently the conservation biology of the federally endangered brackish water species, the tidewater goby, Eucyclogobius newberryi. This species is narrowly adapted to the upper brackish estuarine zone, and is scattered in semi-isolated populations over most of the coast of California. Its biology and systematic relationships contribute to the understanding of the evolution of organisms into the estuarine and brackish water habitat, and the evolution of north Pacific coastal faunas in general. Habitat alteration in much of California has led to a federal endangered listing for this species. Its biology, projected backwards in time, has also contributed to the reconstruction of the original conditions of many California estuaries. This information is very important to the conservation and restoration the coastal marshes in California.

Grants: Research collaborator with Dr. George Dales. Use of fishes by the Harappan Culture Pakistan. Smithsonian Foreign Currency Program, 3 years; Co-principal investigator with L. Barnes, E. D. Mitchell, NSF EAR-7916508, Paleoecology of the Sharktooth Hill Bonebed, 2 years; Research collaborator with Drs. B. Berlin, J. L. Patton (co-principal investigators), NSF BSN-7916746, Field ethnobiological anthropology in the Peruvian Amazon, 3 years; Co-principal investigator with R. J. Lavenberg, NSF DEB-8008088, Development of Ichthyological Resources, 3 years; Co-principal investigator with J. Chovan, A national traveling exhibition on the biology of sharks, NSF Pre-College Education Grant MDR-8751868, 2 years.

Contracts: Freshwater fishes of southern California, survey and report. Calif. Dept. Fish and Game, 1 year, \$75,000. Status of the native freshwater fishes of southern California, including the status of the estuarine tidewater goby, Eucyclogobius newberryi, with recommendations for preserves to maintain their existence. California Department of Fish and Game Contract FG-7455, one year. Cooperative Agreement, National Biological Service and Loyola Marymount University for study of the biology and habitat of tidewater goby on Vandenberg Air Force Base, Santa Barbara County. 1.5 years, \$56,000. Prepare draft recovery plan tidewater goby, U. S. Fish and Wildlife Service, \$5,600. Prepare historical analysis of coastal estuaries, habitat change, and restoration options, mouth Santa Maria River, Santa Barbara County, CA for California Department of Fish and Game Oil Response Team, \$15,000. Co-author, U.S. Fish and Wildlife Service Recovery Plan for Endangered Unarmored Threespine Stickleback. Draft Survey

for the world conservation of fishes, for the International Union for Conservation of Nature and Natural Resources, Switzerland. Biology of the tidewater goby and management of aquatic exotic species, Marine Corps Base Camp Pendleton, CA. 3 years, \$25,000.

Major Publications: 1986-present

1986. Swift, C.R. Gilbert, S.A. Bortone, G.A. Burgess, and R. W. Yerger.

Zoogeography of the freshwater fishes of the southeastern United States: Savannah River to Lake Pontchartrain. pp. 213-265. In: C.H. Hocutt and E.O. Wiley, Eds.,

Zoogeography of North American Freshwater Fishes. Wiley Interscience, N.Y.

1989. Swift, J.S. Nelson, C. Maslow, and T. Stein. 1989. Biology and distribution of the tidewater goby, *Eucyclobius newberryi*, (Pisces, Gobiidae) of California. Nat. Hist. Mus. Los Angeles Co., Contrib. Sci. 404, 19 pp.

1993. Swift, T. H. Haglund, M. Ruiz, and R. Fisher. Status and distribution of the freshwater fishes of southern California. Bull. S. Calif. Acad. Sci., 92(3):101-168.

1996. Chapter 30. Distribution and migration. Pp. 595-630. In: Carl Bond. Biology of Fishes, (textbook) Second Edition. Harcort, Brace, and Co., Philadelphia.

1996. Lafferty, K., R. Swenson, and C. C. Swift. Tidewater goby; endangered species profile. Environmental Biology of Fishes, 46:254.

1999. K. Lafferty, C. C. Swift and R. Ambrose. Extinction and recolonization of populations of the endangered tidewater goby in southern California: a metapopulation? North American Journal of Fisheries Science (In press).

Manuscript in preparation: California Coastal Lagoons, historical and restoration perspectives. Contract for book with University of California Press.

Teaching experience: Comparative Vertebrate Anatomy, Ichthyology-Herpetology, Vertebrate Paleontology, Evolution, Embryology and development (molecular emphasis), Introductory Biology, Research Seminar in Estuarine Ecology, and non majors courses in Natural History of Southern California, Environmental Science, Introductory Biology, and Human Anatomy and Physiology, at Loyola Marymount University, Rio Hondo College, and Mount San Antonio College.

Professional Activities: Various positions, Amer. Soc. Ich. Herp., Cal-Neva Chapter of the Amer. Fish. Soc., S. Calif. Acad. Sci. Secretary, Vice-president, President and Fellow of the Academy. Host committees Los Angeles meetings Amer. Soc. Ich. Herp. (twice), Soc. Vert. Paleo., Cal-Neva Chapter AFS, and S. Calif. Acad. Sci. (three times). Currently Past President (until August, 1999) Cal-Neva Chapter AFS, Member Unarmored Threespine Stickleback Endangered Species Recovery Team (U.S. Fish and Wildlife Service). Research Associate, San Bernardino County Museums.

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February 18,1999

Dave Jacobs UCLA

Dear Dave:

This is to acknowledge our phone conversation of February 17, 1999 regarding our intention to collaborate on studies of the tidewater goby using the microsatellite loci I have identified and designed primers for in the tidewater goby genome. I have identified seven microsatellites and have successfully amplified tidewater goby DNA using five of the primer sets. Three have proven to be polymorphic in the populations I have examined, and the other two have not proven to be polymorphic. The other two primer sets have technical problems which could potentially be solved with some extra work, and they may also prove to be useful. The current study which identified and developed primers for these microsatellite loci have been used to examine the genetic makeup of a sampling of three different populations in Northern California, and was done to fulfill the Thesis requirement for the Masters Program at SJSU.

I am acknowledging my agreement to give these primer sets for these microsatellites which I have identified so they can be used in a grant submitted by Dave Jacobs to NSF, and that he may use them, if the grant is funded, for studies on DNA from fish he has collected at various locations over the range of the California coastline.

Sincerely,

Holly Mendonca

Curriculum Vitae

Holly Lynn Mendonca

390 Madeline Court, Palo Alto, CA 94306 Tel. Work:650.859.2692 Home:650.424.1178

Education

M.S. Biology Projected graduation date: Spring 1999 San Jose State University B.S. Marine Biology May 1989 Summa Cum Laude San Francisco State University

Selected Biology Departmental Honoree, May 1989 Member of Golden Key National Honor Society

Relevant Employment History

SRI International
Pharmaceutical Discovery Division, Cancer Biology Group
333 Ravenswood Avenue
Menlo Park, CA 94025
Supervisor: Dr. K. R. Laderoute
650,859,3080

Title: Biologist II

November 1989 - present

Areas of Expertise

* PCR ... sequencing of DNA

* Affinity and immunoprecipitation kinase assays

* Preparation of nuclear extracts, DNA and protein from cells; preparation of total RNA from cells, plasmid DNA from bacterial cultures

* S blots, N blots with radioactive labeling (32P) of nucleic acid or oligonucleotide probes; Western blots with chemiluminescent detection

- * Transient and stable transfections of mammalian cells using liposomebased methods, calcium phosphate and electroporation
- * Apoptosis assays using Hoescht stain, CHEF gels, and TUNEL assay
- * Immunohistochemistry of cells and tissues...fluorescence microscopy

* Reporter gene activity assay...luciferase and CAT

- * Electrophoresis: agarose gels to analyse DNA or RNA...1-d PAGE (mini and standard) to analyse protein...and others
- * Tissue culture: maintenance and use of mammalian cell lines
- * Computer skills...word processing for Mac and PC... spread sheets... Netscape...

Publications

- K. R. Laderoute, H. L. Mendonca, J. M. Calaoagan, A. M. Knapp, A. J. Giaccia, and P. Stork. Mitogen-activated Protein Kinase Phosphatase-1 (MKP-1) Expression is Induced By Low Oxygen Conditions Found in Solid Tumor Microenvironments. A candidate MKP for the inactivation of hypoxia-inducible SAPK/JNK activity. J. Biol. Chem., 1998 (submitted).
- K. R. Laderoute, J. M. Calaoagan, H. L. Mendonca, W. A. Ausserer, E. Y. Chen, A. J. Giaccia, and R. M. Sutherland. Early responses of SiHa human squamous carcinoma cells to hypoxic signals: Evidence of parallel activation of NF-kB and AP-1 transcriptional complexes. Int. J. Oncol. 8, 875-882, 1996.

- V. K. Langmuir, K. Laderoute, H. L. Mendonca, R. M. Sutherland, T. K. Hei, S. X. Liu, E. J. Hall, M. A. Naylor, and G. E. Adams. Fused pyrazine mono-N-oxides as bioreductive drugs. II. Cytotoxicity in human cells and oncogenicity in a rodent transformation assay. Int. J. Radiat. Oncol. Biol. Phys. 34, 79-84, 1996.
- N. S. Waleh, M. D. Brody, A. M. Knapp, H. L. Mendonca, E. M. Lord, C. J. Koch, K. R. Laderoute, and R. M. Sutherland. Mapping of the vascular endothelial growth factor-producing hypoxic cells in multicellular tumor spheroids using a hypoxic-specific marker. Cancer Res. 55, 6222,6226, 1995.
- V. K. Langmuir and H. L. Mendonca. The combined use of 131I-labeled antibody and the hypoxic cytotoxin SR 4233 in vitro and in vivo. Radiat. Res. 132, 351-358, 1992.
- V. K. Langmuir, B. W. Wessels, H. L. Mendonca, E. D. Yorke, and L. Montilla. Comparisons of micro-TLD dose measurements with predicted dose from 1311-labeled antibody. Med. Phys. 19, 1213-1218, 1992.
- V. K. Langmuir and H. L. Mendonca. The role of radionuclide distribution in the efficacy of 131I-labeled antibody as modeled in multicell spheroids. Antibod. Immunoconjug. Radiopharm. 5, 273-283, 1992.
- V. K. Langmuir, H. L. Mendonca, and D. V. Woo. Comparisons between two monoclonal antibodies that bind to the same antigen but have differing affinities: Uptake kinetics and 125I-antibody efficacy in multicell spheroids. Cancer Res. 52, 4728-4734, 1992.
- V.K. Langmuir, H.L. Mendonca, J.-L. Vanderheyden, and F. M. Su. Comparisons of the efficacy of 186Re and 131I-labeled antibody in multicell spheroids. Int. J. Radiat. Oncol. Biol. Phys. 24, 127-132, 1992

Genetic Identification Services

9552 Topanga Canyon Blvd.
Chatsworth, CA 91311
(818) 718-9600 • (800) 362-4478 • Fax (818) 718-9620
gisemail@genetic-id-services.com
http://www.genetic-id-services.com



February 19, 1999

Dr. David Jacobs
Department of Biology
University of California
Los Angeles, CA 90095

Dear Dr. Jacobs:

This is to confirm GIS' willingness to assist you in producing microsatellite-enriched libraries to support your research in two species of gobies and one species of pipefish. Separate libraries would be produced that are enriched for at least four different motifs, including di-, tri-, and tetranucleotide microsatellites. As necessary, we can also assist in the identification of polymorphic loci through the design and testing of PCR primers developed from microsatellite-containing clones.

GIS has extensive experience in producing and using microsatellite-enriched libraries. At the present time, we have successfully produced libraries for nearly 60 species, including a wide range of both plant and animal taxa. The libraries consist of approximately 8,000 to 10,000 recombinant *E. coli* cells. Depending on the motif and species, enrichments often approach 100% for dinucleotides and 85% for tri- and tetranucleotide motifs. Thus, the libraries generally yield several thousand microsatellite-containing clones. Libraries are generally completed within four to six weeks of receipt of genomic DNA.

Please note that if we are unable to produce a library that is at least 50% enriched, there is no charge for our efforts. Please also note that we've never been unable to produce a highly enriched library in any of the species we've tried, so I'm confident that we will be successful in providing you the loci that you need.

I hope this answers any questions you might have. If you need any more information, please don't hesitate to contact me.

Sincerely yours,

Kenneth Jones

President

The University of Texas Medical Branch at Galveston

School of Medicine Graduate School of Biomedical Sciences School of Allied Health Sciences School of Nursing Marina Biomedical Institute Institute for the Medical Humanities UTMB Hospitals and Clinics



February 19, 1999

Dr. David Jacobs
Department of Biology
University of California
2203 Life Sciences
Los Angeles, CA 90095-1606

Dear Dave:

I have read your proposal on the population ecology and genetics of the Tidewater Goby and other estuarine fish and am very enthusiastic about collaborating with you on the use of microsatellites in defining the genetic structure of populations of these fish. We have been working on various projects dealing with insect population genetics and have been working with microsatellite DNA since 1993. My laboratory is well equipped for analyzing microsatellites with high throughput using automated DNA sequencing (ABI 377) and bio-robotics for handling large numbers of individual PCRs (Beckman Biomek 2000 robotic workstation). We have extensive experience working with software used for analyzing microsatellite gels (Genescan and Genotyper) and in formatting data sets for input into population genetics programs, such as Arlequin.

I would be pleased to host you or your co-workers in my laboratory for the purpose of conducting microsatellite analyses and in providing you with training on the aforementioned equipment and computer software. I enthusiastically agree to act as a collaborator with you. Best of luck on your proposal!

Sincerely.

Gregory C. Lanzaro Associate Professor

Department of Pathology & Center for Tropical Discases

BIOGRAPHICAL SKETCH

Gregory C. Lanzaro

Department of Pathology and Center for Infectious Diseases University of Texas Medical Branch, Galveston, Texas 77555-0609

Education at	nd training	•
Ph.D. 1986	Entomology	University of Florida
M.S. 1978	Entomology	University of Arizona
B.S. 1972	Biology	Kansas State University

Research and Teaching Experience:

7/98-12/90: Post-Doctoral, Department of Entomology, University of California, Davis, C.A.

Population genetics of snowpool Aedes mosquitoes.

1/91-6/93: MacArthur Fellow, Guest Researcher, National Institutes of Health, Laboratory of

Malaria Research. Population genetics of Anopheles gambiae in West Africa.

7/93-8/95: Senior Staff Fellow, MacArthur Fellow, National Institutes of Health, Laboratory

of Malaria Research. Molecular evolution of chromosome inversions in *Anopheles gambiae*; population genetics and molecular genetics of salivary

peptides in the sand fly, Lutzomyia longipalpis.

8/95-8/97: Assistant Professor, Department of Pathology, University of Texas Medical

Branch. Member, W.H.O. Collaborating Center for Tropical Diseases. Researched molecular population genetics of insect vectors of disease.

Taught "Evolution of Infectious Disease" course.

9/97-present: Associate Professor, Department of Pathology, University of Texas Medical

Branch, present Member Center for Infectious Diseases.

12/97: Appointed to Graduate Faculty, The University of Texas Graduate School of

Biomedical Sciences at Galveston

Relevant Publications (11 of 29)

Mutebi, J-P, J.B. Alexander, I. Sherlock, J. Wellington, A.A. Souza, J. Shaw, E.F. Rangel and G.C. Lanzaro. Breeding structure of the sand fly *Lutzomyia longipalpis* (Neiva & Lutz) in Brazil. J. Amer. Soc. Trop. Med. Hyg. In Press.

Lanzaro, G.C., Ribeiro, J.M.C., Warburg, A., Shoemaker, C.B., Lopes, A.H.C.S., Soares, M. and R.G. Titus. Variation in the salivary peptide, maxadilan, from species in the *Lutzomyia* longipalpis complex. Insect Mol. Biol. In Press.

Lanzaro, G.C., Tourè, Y.T., Carnahan, J., Zheng, L., Dolo, G., Traorè, S., Petrarca, V., Vernick, K.D. and C.E. Taylor. Complexities in the genetic structure of *Anopheles gambiae* populations in west Africa as revealed by microsatellite DNA. Proc. Nat. Acad. Sci. USA. 95:14260-14265; 1998.

Yin, H., Mutebi, J. P., Marriott, S. and G.C. Lanzaro. Metaphase karyotypes and chromosome G-banding in members of the *Lutzomyia longipalpis* species complex. Med. Vet. Entomol. 13:1-6; 1998.

Mutebi, J.P., Rowton, E., Herrero, M.V., Ponce, C., Belli, A., Valle. S, and Lanzaro G.C. Genetic variability among Central American field populations of the sand fly, *Lutzomyia*

- (Lutzomyia) longipalpis (Lutz & Meiva) (Diptera: Psychodidae). J. Med. Entomol. 35: 169-174, 1998.
- Lanzaro G.C., Alexander, B., Mutebi, J.P., Montoya-Lerma, J. and Warburg, A. Genetic variation among natural and laboratory colony populations of *Lutzomyia longipalpis* from Columbia. Mem. Oswaldo Cruz. 93: 65-70, 1997.
- Rongnoparut, P., Yaicharoen, S., Sirichotpakorn, N., Rattanarithikul, R., Lanzaro, G.C., and Linthicum, K. Microsatellite polymorphism in *Anopheles maculatus*, a malaria vector in Thailand. Am. J. Trop. Med. Hyg. 55: 589-594, 1996.
- Lanzaro, G.C., Warburg, A. Genetic variability in phlebotomine sand flies: Possible implications for the epidemiology of leishmaniasis. Parasitol Today 11: 151-154, 1995.
- Mathiopoulos, K.D., Lanzaro, G.C. Distribution of genetic diversity in relation to chromosomal inversions in the malaria mosquito, *Anopheles gambiae*. J Mol Evol 40: 578-584, 1995.
- Lanzaro, G.C., Tourè, Y., Zheng, L., Kafatos, F., and Vernick, K. Microsatellite DNA and isozyme variability in a West African population of *Anopheles gambiae*. Insect Mol Biol 4: 105-112, 1995.
- Warburg, A., Saravia, E., Lanzaro, G.C., Titus, R.G., and Neva, F. Saliva of *Lutzomyia longipalpis* sibling species differs in its composition and capacity to enhance leishmaniasis. Trans Roy Phil Soc 345:†261-267, 1994.

Most Recent Invited Presentations (Since 1995)

- Keystone Symposia on Molecular & Cellular Biology, Taos, New Mexico, January 9-15, 1998. "Complexities in the genetic structure of vector populations: *Anopheles gambiae* in West Africa" In Symposium: "Toward the Genetic Manipulation of Insects".
- Annual Meeting, Society for Vector Ecology. College Station, Texas, October 4-7, 1998. "Pitfalls and promise of microsatellite DNA in vector ecology and systematics" In Symposium: "Molecular Methods in Vector Systematics".
- Annual Meeting, American Society of Tropical Medicine & Hygiene. Orlando, Florida, December†7-11, 1997. American Committee of Medical Entomology Symposium. "Identification of the real vector: sibling species complexes".
- Second International Congress of Vector Ecology, Orlando, Florida, October 19-24, 1997. "The distribution of genetic variation in *Anopheles gambiae*: from genomes to populations".
- Entomological Society of America, Annual Meeting, Louisville, Kentucky, December 8-12, 1996. "Highlights in Medical Entomology, 1995-96".
- XX International Congress of Entomology, Florence, Italy, August 25-31, 1996. "Variation in the vasodilatory activity of saliva among populations of the sand fly, *Lutzomyia longipalpis*."
- MacArthur Foundation, Network on the Biology of Parasite Vectors, Institute, Fort Collin, Colorado, June, 1995. "Microsatellite DNA for the study of the population genetics of Anopheles gambiae".

U.S. Department of the Interior

Certifications Regarding Debarment, Suspension and Other Responsibility Matters, Drug-Free Workplace Requirements and Lobbying

Persons signing this form should refer to the regulations referenced below for complete instructions:

Certification Regarding Debarment, Suspension, and Other Responsibility Matters - Primary Covered Transactions - The prospective primary participant further agrees by submitting this proposal that it will include the clause titled, "Certification Regarding Debarment, Suspension, Ineligibility and Voluntary Exclusion - Lower Tier Covered Transaction," provided by the department or agency entering into this covered transaction, without modification, in all lower tier covered transactions and in all solicitations for lower tier covered transactions. See below for language to be used or use this form certification and sign. (See Appendix A of Subpart D of 43 CFR Part 12.)

Certification Regarding Debarment, Suspension, Ineligibility and Voluntary Exclusion - Lower Tier Covered Transactions - (See Appendix B of Subpart D of 43 CFR Part 12.)

Certification Regarding Drug-Free Workplace Requirements - Alternate I. (Grantees Other Than Individuals) and Alternate II. (Grantees Who are Individuals) - (See Appendix C of Subpart D of 43 CFR Part 12)

Signature on this form provides for compliance with certification requirements under 43 CFR Parts 12 and 18. The certifications shall be treated as a material representation of fact upon which reliance will be placed when the Department of the Interior determines to award the covered transaction, grant, cooperative agreement or loan.

PART A: Certification Regarding Debarment, Suspension, and Other Responsibility Matters-Primary Covered Transactions

CHECK____IF THIS CERTIFICATION IS FOR A PRIMARY COVERED TRANSACTION AND IS APPLICABLE.

- (1) The prospective primary participant certifies to the best of its knowledge and belief, that it and its principals:
 - (a) Are not presently debarred, suspended, proposed for debarment, declared ineligible, or voluntarily excluded by any Federal department or agency;
 - (b) Have not within a three-year period preceding this proposal been convicted of or had a civil judgment rendered against them for commission of fraud or a criminal offense in connection with obtaining, attempting to obtain, or performing a public (Federal, State or local) transaction or contract under a public transaction; violation of Federal or State antitrust statutes or commission of embezzlement, theft, forgery, bribery, falsification or destruction of records, making false statements, or receiving stolen property;
 - (c) Are not presently indicted for or otherwise criminally or civilly charged by a governmental entity (Federal, State or local) with commission of any of the offenses enumerated in paragraph (1)(b) of this certification; and
 - (d) Have not within a three-year period preceding this application/proposal had one or more public transactions (Federal, State or local) terminated for cause or default.
- (2) Where the prospective primary participant is unable to certify to any of the statements in this certification, such prospective participant shall attach an explanation to this proposal.

PART B: Certification Regarding Debarment, Suspension, Ineligibility and Voluntary Exclusion - Lower Tier Covered Transactions

CHECK IF THIS CERTIFICATION IS FOR A LOWER TIER COVERED TRANSACTION AND IS APPLICABLE.

- (1) The prospective lower tier participant certifies, by submission of this proposal, that neither it nor its principals is presently debarred, suspended, proposed for debarment, declared ineligible, or voluntarily excluded from participation in this transaction by any Federal department or agency.
- (2) Where the prospective lower tier participant is unable to certify to any of the statements in this certification, such prospective participant shall attach an explanation to this proposal.

PART C: Certification Regarding Drug-Free Workplace Requirements

CHECK X IF THIS CERTIFICATION IS FOR AN APPLICANT WHO IS NOT AN INDIVIDUAL.

Alternate I. (Grantees Other Than Individuals)

- A. The grantee certifies that it will or continue to provide a drug-free workplace by:
 - (a) Publishing a statement notifying employees that the unlawful manufacture, distribution, dispensing, possession, or use of a controlled substance is prohibited in the grantee's workplace and specifying the actions that will be taken against employees for violation of such prohibition;
 - (b) Establishing an ongoing drug-free awareness program to inform employees about--
 - (1) The dangers of drug abuse in the workplace;
 - (2) The grantee's policy of maintaining a drug-free workplace;
 - (3) Any available drug counseling, rehabilitation, and employee assistance programs; and
 - (4) The penalties that may be imposed upon employees for drug abuse violations occurring in the workplace;
 - (c) Making it a requirement that each employee to be engaged in the performance of the grant be given a copy of the statement required by paragraph (a);
 - (d) Notifying the employee in the statement required by paragraph (a) that, as a condition of employment under the grant, the employee will --
 - (1) Abide by the terms of the statement; and
 - (2) Notify the employer in writing of his or her conviction for a violation of a criminal drug statute occurring in the workplace no later than five calendar days after such conviction;
 - (e) Notifying the agency in writing, within ten calendar days after receiving notice under subparagraph (d)(2) from an employee or otherwise receiving actual notice of such conviction. Employers of convicted employees must provide notice, including position title, to every grant officer on whose grant activity the convicted employee was working, unless the Federal agency has designated a central point for the receipt of such notices. Notice shall include the identification number(s) of each affected grant;
 - (f) Taking one of the following actions, within 30 calendar days of receiving notice under subparagraph (d)(2), with respect to any employee who is so convicted --
 - (1) Taking appropriate personnel action against such an employee, up to and including termination, consistent with the requirements of the Rehabilitation Act of 1973, as amended; or
 - (2) Requiring such employee to participate satisfactorily in a drug abuse assistance or rehabilitation program approved for such purposes by a Federal, State, or local health, law enforcement, or other appropriate agency;
 - (g) Making a good faith effort to continue to maintain a drug-free workplace through implementation of paragraphs (a) (b), (c), (d), (e) and (f).
- B. The grantee may insert in the space provided below the site(s) for the performance of work done in connection with the specific grant:

specific grant.	
Place of Performance (Street address, city, county, state, zip code)	
621 Young Drive, South	

Los Angeles, CA 90095

Check____if there are workplaces on files that are not identified here.

PART D: Certification Regarding Drug-Free Workplace Requirements

CHECK___IF THIS CERTIFICATION IS FOR AN APPLICANT WHO IS AN INDIVIDUAL.

Alternate II. (Grantees Who Are Individuals)

- (a) The grantee certifies that, as a condition of the grant, he or she will not engage in the unlawful manufacture, distribution, dispensing, possession, or use of a controlled substance in conducting any activity with the grant;
- (b) If convicted of a criminal drug offense resulting from a violation occurring during the conduct of any grant activity, he or she will report the conviction, in writing, within 10 calendar days of the conviction, to the grant officer or other designee, unless the Federal agency designates a central point for the receipt of such notices. When notice is made to such a central point, it shall include the identification number(s) of each affected grant.

PART E: Certification Regarding Lobbying Certification for Contracts, Grants, Loans, and Cooperative Agreements

CHECK_X IF CERTIFICATION IS FOR THE AWARD OF ANY OF THE FOLLOWING AND THE AMOUNT EXCEEDS \$100,000: A FEDERAL GRANT OR COOPERATIVE AGREEMENT; SUBCONTRACT, OR SUBGRANT UNDER THE GRANT OR COOPERATIVE AGREEMENT.

CHECK___IF CERTIFICATION FOR THE AWARD OF A FEDERAL LOAN EXCEEDING THE AMOUNT OF \$150,000, OR A SUBGRANT OR SUBCONTRACT EXCEEDING \$100,000, UNDER THE LOAN.

The undersigned certifies, to the best of his or her knowledge and belief, that:

- (1) No Federal appropriated funds have been paid or will be paid, by or on behalf of the undersigned, to any person for influencing or attempting to influence an officer or employee of an agency, a Member of Congress, and officer or employee of Congress, or an employee of a Member of Congress in connection with the awarding of any Federal contract, the making of any Federal grant, the making of any Federal loan, the entering into of any cooperative agreement, and the extension, continuation, renewal, amendment, or modification of any Federal contract, grant, loan, or cooperative agreement.
- (2) If any funds other than Federal appropriated funds have been paid or will be paid to any person for influencing or attempting to influence an officer or employee of any agency, a Member of Congress, an officer or employee of Congress, or an employee of a Member of Congress in connection with this Federal contract, grant, loan, or cooperative agreement, the undersigned shall complete and submit Standard Form-LLL, "Disclosure Form to Report Lobbying," in accordance with its
- (3) The undersigned shall require that the language of this certification be included in the award documents for all subawards at all tiers (including subcontracts, subgrants, and contracts under grants, loans, and cooperative agreements) and that all subrecipients shall certify accordingly.

This certification is a material representation of fact upon which reliance was placed when this transaction was made or entered into. Submission of this certification is a prerequisite for making or entering into this transaction imposed by Section 1352, title 31, U.S. Code. Any person who fails to file the required certification shall be subject to a civil penalty of not less than \$10,000 and not more than \$100,000 for each such failure.

As the authorized certifying official, I hereby certify that the above specified certifications are true.

SIGNATURE OF AUTHORIZED CERTIFYING OFFICIAL

Hardy Dhillon, Contract and Grant Officer

TYPED NAME AND TITLE

DATE

State of Charlettina	DEPARTMENT OF WALE? RESOURCES	the semantee. Agency
		Agreement No.
	•	Lixhipit

STANDARD CLAUSES -INTERAGENCY AGREEMENTS

Audit Clause. For contracts in excess of \$10,000, the contracting parties shall be subject to the examination and audit of the State Auditor for a period of three years after final payment under the contract (Covernment Code Section 8546.7).

Availability of Funds. Work to be performed under this contract is subject to availability of funds through the Studies normal budget process.

Interrigency Phyment Clause. For services provided under this accomment, charges will be computed in containing with State Administrative Manual Section 5 (32 and 8757-1).

cermination Clause. Fuller State agency may tenomed this contract opins 10 division written not be the State agency providing the services size. The remotured for all renominal expenses mentred up to the late of tenometric.

Severability. If any provision of this Agreement is held invalid or unenforceable by any court of final orientation, it is the intent of the parties that all other provisions of this Agreement by construed to remain tools yould, enforceable, and building on the parties.

Y2K Language. The Contractor warrants and represents that the goods or services sold, leased, or humised to the State of California, its accretics, or its political subdivisions, pursuout to this Agreement are. Year 2000 compliant: For purposes of this Agreement, a good or service is year 2000 compliant if it will continue to fully function before, at, and after the Year 2000 without interruption and, if applicable, with full ability to accurately and mambiguously process, display, compare, calculate, manipulate, and otherwise utilize date information. This warranty and representation supersedes all warranty disclaimers and firmtations and all limitations on hability provided by or through the Contractor.

DWR 4187 (REV 1/99)

4/7/99 3:33 P

OMB Approval No. 0348-0043 APPLICATION FOR 2. DATE SUBMITTED Applicant Identifier FEDERAL ASSISTANCE TYPE OF SUBMISSION 3. DATE RECEIVED BY STATE State Applicant Identifier Application Preapplication Construction Construction 4. DATE RECEIVED BY FEDERAL AGENCY Federal Identifier X Non-Construction Non-Construction 5. APPLICANT INFORMATION Legal Name: Organizational Unit: The Regents of the University of California Organismic Bio., Ecology & Evolution Address (give city, county, state, and zip code): Name and telephone and E-mail number of the person to be contacted on matters involving this application (give area code) Box 951406 1400 Ueberroth Hardy Dhillon, (310) 825-0965; hdhillon@srnet.ucla.edu Los Angeles, Ca. 90095-1406 6. EMPLOYER IDENTIFICATION NUMBER (EIN): 7. TYPE OF APPLICANT: (enter appropriate letter in box) 6 0 0 State H. Independent School Dist. B. County State Controlled Institution of Higher Learning 8. TYPE OF APPLICATION: Ċ. Municipal Private University D. Township Indian Tribe **⋉** New Continuation Revision Individual E. Interstate Intermunicipal Profit Organization If Revision, enter appropriate letter(s) in box(es): Special District N. Other (Specify) A. Increase Award B. Decrease Award C. Increase Duration 9. NAME OF FEDERAL AGENCY: D. Decrease Duration Other (specify): U.S. Department of the Interior 10. CATALOG OF FEDERAL DOMESTIC 11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT: ASSISTANCE NUMBER: A Phylogeographic and Microsattelite Study of Pacific TITLE: CALFED Bay-Delta program Coast Estuarian Restricted Fish 12. AREAS AFFECTED BY PROJECT (cities, counties, states, etc.): All coastal California Counties 13. PROPOSED PROJECT: 14. CONGRESSIONAL DISTRICTS OF: Start Date **Ending Date** a. Applicant b. Project 29th Various 10/01/99 09/30/02 15. ESTIMATED TOTAL PROJECT FUNDING: 16. IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS? a. YES. THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON: a. Federal \$ 106,098 .00 b. Applicant \$ 19,872 DATE c. State \$.00 b. NO. X PROGRAM IS NOT COVERED BY E.O. 12372 d. Local ŧ .00 OR PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW e. Other .00 f. Program Income 17. IS THE APPLICANT DELINQUENT ON ANY FEDERAL DEBT? .00 g. TOTAL ... Yes If "Yes," attach an explanation. 125,970 .00 18. TO THE BEST OF MY KNOWLEDGE AND BELIEF, ALL DATA IN THIS APPLICATION/PREAPPLICATION ARE TRUE AND CORRECT. THE DOCUMENT HAS BEEN DULY AUTHORIZED BY THE GOVERNING BODY OF THE APPLICANT AND THE APPLICANT WILL COMPLY WITH THE ATTACHED ASSURANCES IF THE ASSISTANCE IS AWARDED. b. Title Contract and Grant Officer a. Typed Name of Authorized Representative Telephone number Hardy Dhillon (310) 825-0695 d. Signature of Authorized Representative e. Date Signed

ASSURANCES—NON-CONSTRUCTION PROGRAMS

Public reporting burden for this collection of information is estimated to average 45 minutes per response, including time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding the burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to the Office of Management and Budget. Paperwork Reduction Project (0348-0043), Washington, DC 20503.

PLEASE DO NOT RETURN YOUR COMPLETED FORM TO THE OFFICE OF MANAGEMENT AND BUDGET, SEND IT TO THE ADDRESS PROVIDED BY THE SPONSORING AGENCY.

Note:

Certain of these assurances may not be applicable to your project or program. If you have questions please contact the awarding agency. Further, certain Federal awarding agencies may require applicants to certify to additional assurances. If such is the case, you will be notified.

As the duly authorized representative of the applicant I certify that the applicant:

- 1. Has the legal authority to apply for Federal assistance, and the institutional, managerial and financial capability (including funds sufficient to pay the non-Federal share of project costs) to ensure proper planning, management and completion of the project described in this application.
- 2. Will give the awarding agency, the Comptroller General of the United States, and if appropriate, the State, through any authorized representative, access to and the right to examine all records, books, papers, or documents related to the award; and will establish a proper accounting system in accordance with generally accepted accounting standards or agency directives.
- Will establish safeguards to prohibit employees from using their positions for a purpose that constitutes or presents the appearance of personal or organizational conflict of interest, or personal gain.
- 4. Will initiate and complete the work within the applicable time frame after receipt of approval of the awarding agency.
- 5. Will comply with the Intergovernmental Personnel Act of 1970 (42 U.S.C. §§ 4728-4763) relating to prescribed standards for merit systems for programs funded under one of the nineteen statutes or regulations specified in Appendix A of OPM's Standards for a Merit System of Personnel Administration (5 C.F.R. 900, Subpart F).
- 6. Will comply with all Federal statutes relating to nondiscrimination. These include but are not limited to: (a) Title VI of the Civil Rights Act of 1964 (P.L. 88-352) which prohibits discrimination on the basis of race, color or national origin; (b) Title IX of the Education Amendments of 1972, as amended (20 U.S.C. §§ 1681-1683, and 1685-1686), which prohibits discrimination on the basis of sex; (c) Section 504 of the Rehabilitation

- Act of 1973, as amended (29 U.S.C. § 794), which prohibits discrimination on the basis of handicaps; (d) the Age Discrimination Act of 1975, as amended (42 U.S.C. §§ 6101-6107), which prohibits discrimination on the basis of age; (e) the Drug Abuse Office and Treatment Act of 1972 (P.L. 92-2S5), as amended, relating to nondiscrimination on the basis of drug abuse; (f) the Comprehensive Alcohol Abuse and Alcoholism Prevention, Treatment and Rehabilitation Act of 1970 (P.L. 91-616), as amended, relating to nondiscrimination on the basis of alcohol abuse or alcoholism: (g) §§ 523 and 527 of the Public Health Service Act of 1912 (42 U.S.C. 290 dd-3 and 290 ee-3), as amended, relating to confidentiality of alcohol and drug abuse patient records; (h) Title VIII of the Civil Rights Act of 1968 (42 U.S.C. § 3601 et seq.), as amended, relating to non-discrimination in the sale, rental or financing of housing; (i) any other nondiscrimination provisions in the specific statute(s) under which application for Federal assistance is being made; and (j) the requirements of any other nondiscrimination statute(s) which may apply to the application.
- 7. Will comply, or has already complied, with the requirements of Titles II and 111 of the Uniform Relocation Assistance and Real Property Acquisition Policies Act of 1970 (P.L. 91-646) which provide for fair and equitable treatment of persons displaced or whose property is acquired as a result of Federal or federally assisted programs. These requirements apply to all interests in real property acquired for project purposes regardless of Federal participation in purchases.
- 8. Will comply with the provisions of the Hatch Act (5 U.S.C. §§ 1501-1508 and 7324-7328) which limit the political activities of employees whose principal employment activities are funded in whole or in part with Federal funds.

- 9. Will comply, as applicable, with the provisions of the Davis-Bacon Act (40 U.S.C. §§ 276a to 276a7), the Copeland Act (40 U.S.C. § 276c and 18 U.S.C. §§ 874), and the Contract Work Hours and Safety Standards Act (40 U.S.C. §§ 327-33.3), regarding labor standards for federally assisted construction subagreements.
- 10. Will comply, if applicable, with flood insurance purchase requirements of Section 102(a) of the Flood Disaster Protection Act of 1973 (P.L. 93284) which requires recipients in a special flood hazard area to participate in the program and to purchase flood insurance if the total cost of insurable construction and acquisition is \$10,000 or more.
- 11. Will comply with environmental standards which may be prescribed pursuant to the following: (a) institution of environmental quality control measures under the National Environmental Policy Act of 1969 (P.L. 91-190) and Executive Order (EO) 11514; (b) notification of violating facilities pursuant to EO 11738; (c) protection of wetlands pursuant to EO 11990; (d) evaluation of flood hazards in floodplains in accordance with EO 11988; (e)assurance of project consistency with the approved State management program developed under the Coastal Management Act of 1972 (16 U.S.C. §§ 1451 et seq.); (f) conformity of Federal actions to State (Clear Air) Implementation Plans under Section 176(c) of the Clear Air Act of 1955, as amended (42 U.S.C. § 7401 et seq.); (g) protection of underground sources of drinking water under the Safe Drinking Water Act of 1974, as amended, (P.L. 93-523); and (h) protection of endangered species under the Endangered Species Act of 1973, as amended, (P.L. 93-205).

- 12. Will comply with the Wild and Scenic Rivers Act of 1968 (16 U.S.C. §§ 1271 et seq.) related to protecting components or potential components of the national wild and scenic rivers system.
- 13. Will assist the awarding agency in assuring compliance with Section 106 of the National Historic Preservation Act of 1966, as amended (16 U.S.C. 470), EO 11593 (identification and protection of historic properties), and the Archaeological and Historic Preservation Act of 1974 (16 U.S.C. 469a-1 et seq.).
- 14. Will comply with P.L. 93-348 regarding the protection of human subjects involved in research, development, and related activities supported by this award of assistance.
- 15. Will comply with the Laboratory Animal Welfare Act of 1966 (P.L. 89-544, as amended, 7 U.S.C. 2131 et seq.) pertaining to the care, handling, and treatment of warm blooded animals held for research, teaching, or other activities supported by this award of assistance.
- 16. Will comply with the Lead-Based Paint Poisoning Prevention Act (42 U.S.C. §§ 4801 et seq.) which prohibits the use of lead based paint in construction or rehabilitation of residence structures.
- 17. Will cause to be performed the required financial and compliance audits in accordance with the Single Audit Act of 1995 or OMB Circular No. A-133, Audits of Institutions of Higher Learning and other Non-profit Institutions.
- 18. Will comply with all applicable requirements of all other Federal laws, executive orders, regulations and policies governing this program.

SIGNATURE OF AUTHORIZED CERTIFYING OFFICIAL	TITLE
Hody Lak	Hardy Dhillon Contract and Grant Officer
APPLICANT ORGANIZATION	DATE SUBMITTED
The Regents of the University of California Box 951406, 1401 Ueberroth Los Angeles, CA 90095-1406	4/15/99